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CLINICAL & BASIC RESEARCH

Association of Higher *Defensin β-4* Genomic Copy Numbers with Behçet's Disease in Iraqi Patients

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الترابط بين أعداد النسخ الجينومية المرتفعة لبروتين ديفنسين بيتا 4 (*DEFB4*) وبين داء بهجت عند المرضى العراقيين

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abstract: ***Objectives:*** Behçet's disease (BD) is an immune-mediated small vessel systemic vasculitis. Human β-defensins are antimicrobial peptides associated with many inflammatory diseases and are encoded by the *β-defensin* family of multiple-copy genes. However, their role in BD necessitates further investigation. The aim of the present study was to investigate the possible association of BD in its various clinical forms with *defensin β-4* (*DEFB4*) genomic copy numbers. ***Methods:*** This case-control study was conducted from January to September 2011 and included 50 control subjects and 27 unrelated Iraqi BD patients registered at Baghdad Teaching Hospital, Bagdad, Iraq. Copy numbers of the *DEFB4* gene were determined using the comparative cycle threshold method by duplex real-time polymerase chain reaction technology at the Department of Dermatology of Jena University Hospital, Jena, Germany. ***Results:*** *DEFB4* genomic copy numbers were significantly higher in the BD group compared to the control group ($P = 0.010$). However, no statistically significant association was found between copy numbers and clinical variables within the BD group. ***Conclusion:*** The *DEFB4* copy number polymorphism may be associated with BD; however, it is not associated with different clinical manifestations of the disease.

Keywords: Behçet Disease; beta-Defensins; Genetic Polymorphisms; Gene Copy Numbers; Iraq.

الملخص: الهدف: داء بهجت هو التهاب مناعي جهازي يصيب الأوعية الدموية الصغيرة. وبيتا ديفنسين هي بيبتيدات مضادة للميكروبات ترتبط بكثير من الأمراض الالتهابية، وتكون مشفرة في عائلة بيتا ديفينسين المكونة من مورثات (جينات) متعددة النسخ. غير أن الدور الذي قد تلعبه تلك المورثات في داء بهجت ما زال يحتاج لمزيد من الدراسة. ويهدف هذا البحث لدراسة الترابط الممكن بين داء بهجت بصوره الإكلينيكية المختلفة وبين أعداد النسخة الجينومية *DEFB4*. **الطريقة:** أجريت هذه الدراسة الاستعادية من يناير إلى سبتمبر 2011م وشملت 50 فردا صحيحا (مجموعة ضابطة) و27 مريضا عراقيا (ليسوا بأقرباء) مصابين بداء بهجت من المسجلين بمستشفى بغداد التعليمي في العراق. وحددت أرقام مورثات *DEFB4* بتقنية تفاعل التسلسل البلومريزي بقسم الأمراض الجلدية في مستشفى الجامعة بجينا في ألمانيا. **النتائج:** وجد أن أرقام النسخة الجينومية *DEFB4* كانت أعلى معنويا عند مرضى داء بهجت عند المقارنة مع المجموعة الضابطة (0.010 = P). غير أنه لم تكن هنالك أي فروقات معنوية بين نسخ أرقام مورثات *DEFB4* والأعراض الإكلينيكية للمصابين بالمرض. **الخلاصة:** هنالك ارتباط بين تعدد أشكال أرقام *DEFB4* وبين داء بهجت، رغم عدم وجود أي ارتباط لها مع الأعراض الإكلينيكية.

مفتاح الكلمات: داء بهجت؛ بيتا ديفنسين؛ تعدد الأشكال الوراثية؛ أعداد نسخ المورثات؛ العراق.

Advances in Knowledge

- *As part of its genetic pathogenesis, Behçet's disease (BD) may be associated with the presence of a high number of human defensin β-4 (DEFB4) genomic copy numbers, as observed among this sample of unrelated Iraqi BD patients.*
- *The DEFB4 genomic copy numbers in the healthy Iraqi control group were comparable to those observed in other populations.*

Application to Patient Care

- *BD has neither a specific diagnostic test nor a targeted course of therapy. As such, exploring the genetic basis of this autoimmune disease could improve understanding of its pathological mechanisms and promote the development of a more accurate diagnostic and therapeutic approach.*

Behçet's disease (BD) is a chronic autoimmune disease characterised by multiple inflammatory lesions of the orogenital mucosa, eyes and skin with additional involvement of other organs in the body.[1] BD is prevalent in the Mediterranean region and eastern Asia;[2] Iraq is also known to have a relatively high prevalence of the disease.[3] Although the precise pathogenesis

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of BD remains unknown, genetic, immunological and environmental factors have been suggested to contribute to its development.[2] A genetic basis for BD is supported by its high prevalence in certain geographic areas, the familial aggregation of cases and a strong association with human leukocyte antigen-B51.[4,5] Moreover, single nucleotide polymorphism studies have revealed associations with several genes, including *interferon regulatory factor 1* and *tumour necrosis factor-α* (*TNF-α*).[6,7]

Human β-defensins (hBDs) are antimicrobial peptides expressed by various epithelial tissues. They are encoded by the multiple-copy *β-defensin* gene family, copies of which are clustered on the short arm of chromosome eight, including the human *defensin β-2* gene synthesised by *defensin β-4* (*DEFB4*).[8] This *β-defensin* cluster has a genomic copy number of between two and 12 copies per genome, with a mode of four copies.[9] Such variation in genomic copy numbers may play a role in innate immunity; research has shown that hBD-2 has the ability to function as a chemokine that attracts inflammatory cells, such as dendritic cells and T lymphocytes, to the inflamed tissues.[10,11] Thus, it is possible that variations in the *DEFB4* gene might influence an individual's susceptibility to immune-mediated diseases. The antimicrobial and proinflammatory nature of these β-defensins has led many researchers to hypothesise that variations in gene dosage may affect the pathogenesis of many immunological diseases.[12] Hence, the present study aimed to investigate genomic copy numbers of *DEFB4* in Iraqi BD patients in comparison with normal healthy controls. Various clinical manifestations of BD were also studied in association with *DEFB4* genomic copy numbers.

Methods

This case-control study was conducted between January and September 2011 and included all unrelated Iraqi patients registered at the BD Clinic in Baghdad Teaching Hospital, Baghdad, Iraq (n = 27), and randomly selected healthy control subjects (n = 50). All of the BD patients fulfilled the International Study Group criteria for a diagnosis of BD, which requires the presence of recurrent oral ulcers plus at least two of the following symptoms: recurrent genital ulcerations, typical defined eye or skin lesions or a positive skin pathergy test (a skin hypersensitivity reaction to a non-specific physical insult).[13,14] Any patient who did not fulfil these criteria was excluded from the study. The control group included healthy individuals who donated their blood to the central blood bank after confirmation that they were free from any symptoms or signs suggestive of BD.

Blood was collected from all of the subjects and genomic DNA was extracted from peripheral blood mononuclear cells by a standard phenol-chloroform method at the Department of Pathology in Baghdad Medical College, Baghdad. Samples were then stored at -20 °C and delivered to the Department of Dermatology at Jena University Hospital, Jena, Germany. DNA concentrations and quality were then determined with a NanoDrop 1000 spectrophotometer (Nanodrop Technologies Inc., Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) using the following settings: optical density (OD) at 260/280 ~1.8 and OD at 260/230 = 2.0–2.2. Samples were anonymised and the genomic copy number analysis was performed by individuals who were blinded to the relevant clinical information.

Diploid *DEFB4* genomic copy numbers (GenBank: AF040153.1)[15] were determined by a duplex real-time polymerase chain reaction (PCR) technique as described by Jaradat *et al*.[16] Briefly, PCR was performed in 96-Well Microplates (Applied Biosystems, Thermo Fisher Scientific Inc.) in a 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc.). All assays were performed in quadruplicate. Each plate included templates for genomic DNA, negative controls and NA07048HM calibrator DNA (Coriell Institute for Medical Research, Camden, New Jersey, USA) at four copies per genome. Each well contained a 20 µL reaction mixture including 4 µL of genomic DNA (5 ng/µL), 10 µL of TaqMan® Genotyping Master Mix (Thermo Fisher Scientific Inc.) 1 µL of TaqMan® Copy Number Assay Mix (Applied Biosystems, Thermo Fisher Scientific Inc.), 1 µL of TaqMan® Copy Number RNase P Reference Assay mix (Applied Biosystems, Thermo Fisher Scientific Inc.) and 4 µL of nuclease-free water (Ambion® GmbH, Thermo Fisher Scientific Inc.).

The reaction conditions were as follows: 95 °C for 10 minutes for initial denaturation and enzyme activation followed by 40 cycles of 95 °C for 15 seconds and 60 °C for one minute. Cycle threshold values were calculated using the ABI PRISM® 7700 Sequence Detection System, Version 1.3 (Applied Biosystems, Thermo Fisher Scientific Inc.). Relative quantitation was performed using Copy CallerTM Software (Applied Biosystems) to estimate the genomic copy number in each sample according to the comparative cycle threshold method (ΔΔCt).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 16.0, (IBM Corp., Chicago, Illinois, USA).

Table 1: Demographic variables of patients with Behçet's disease in comparison to healthy controls in Baghdad, Iraq (N = 77)

Variable	Control group (n = 50)	BD group (n = 27)	*P* value
Mean age in years ± SD	34.20 ± 8.88	36.39 ± 10.00	0.33*
Gender, n			0.57†
Female	20	9	
Male	30	18	

BD = Behçet's disease; SD = standard deviation.
**Calculated using the unpaired Student's t-test. †Calculated using the Pearson's Chi-squared test.*

Data were presented as the mean estimated copy numbers of *DEFB4*. Each single genomic copy number estimate was rounded to the nearest integer number. Comparisons between the study groups were performed using an unpaired Student's t-test to assess the differences in the means ± standard deviation of *DEFB4* genomic copy number values. Pearson's Chi-squared test was used to analyse categorical data. All *P* values were two-sided and statistical significance was set at $P \leq 0.05$.

This study was approved by the Ethical Committee of the Iraqi Board for Medical Specializations (approval #3) according to the principles of the Declaration of Helsinki. All subjects gave informed consent for inclusion in this study.

Results

A total of 27 BD patients were recruited along with 50 healthy controls. No significant difference in age ($P = 0.33$) or gender ($P = 0.57$) was found between the groups [Table 1]. The *DEFB4* integer genomic copy number range was 3–7 copies per diploid genome in BD cases compared with 2–6 copies in the control group. The mean was 4.44 ± 1.15 copies versus 3.80 ± 0.92 copies in the BD and control groups, respectively. The *DEFB4* genomic copy number was significantly higher in BD cases than in controls ($P = 0.01$) [Figure 1]. No association was found between the clinical presentations of BD and *DEFB4* genomic copy numbers [Table 2].

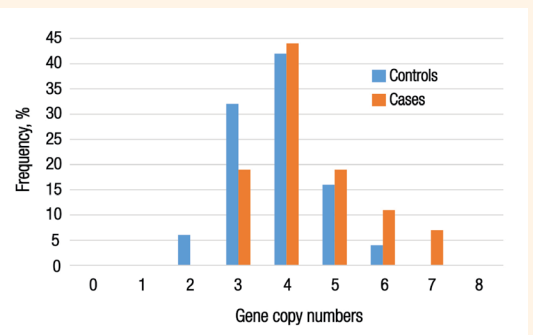

Figure 1: Frequency of *defensin β-4* (*DEFB4*) genomic copy numbers among Iraqi patients with Behçet's disease (n = 27) in comparison to healthy controls (n = 50). The mean *DEFB4* genomic copy number was significantly higher in the BD group ($P = 0.01$).

Table 2: *Defensin β-4* genomic copy numbers by clinical variables among patients with Behçet's disease in Baghdad, Iraq (N = 27)

Clinical variable	Present		Absent		*P* value*
	n	Mean GCN ± SD	n	Mean GCN ± SD	
Genital ulcer	17	4.39 ± 9.94	10	4.18 ± 1.19	0.21
Ocular lesion	7	4.15 ± 0.20	20	4.36 ± 1.17	0.64
Skin lesion	18	4.21 ± 1.03	9	4.46 ± 0.09	0.47
Arthritis	23	4.53 ± 1.09	4	4.46 ± 1.02	0.72
Positive skin pathergy test	20	4.47 ± 1.11	7	3.79 ± 0.10	1.22

GCN = gene copy number; SD = standard deviation.
**Determined using the unpaired Student's t-test.*

Discussion

High *DEFB4* genomic copy number values have been reported in patients with psoriasis and chronic obstructive pulmonary disease.[17,18] Previous studies have also demonstrated increased *DEFB4* messenger ribonucleic acid expression of these copy number variations on the cellular level and in the mucosal tissues of the colon, upper airways and psoriatic skin.[9,18–20] In a large Chinese sample, significantly higher *DEFB4* gene numbers were observed among patients with systemic lupus erythematosus and anti-neutrophil cytoplasmic antibody-associated small vasculitis with a mean genomic copy number of 3.98 and 4.05, respectively.[21] Jansen *et al.* presented evidence that both genomic copy number and pro-inflammatory cytokines affect the biological levels of hBD-2.[20]

BD is a good example of a disease rooted in immunological disturbance mediated by cytokines derived from T-helper lymphocytes, such as TNF-α, which acts as a mediator in the initiation and propagation of BD.[22] In this context, TNF-α is a common inducer of *DEFB4* expression.[18] Possible mechanisms underlying the uncontrolled inflammatory response seen in BD to infection or other environmental triggers are either a high basal level of hBD-2 occurring in

genetically susceptible individuals with high *DEFB4* genomic copy numbers or high levels of hBD-2 induced through the stimulation of TNF-α. It is notable that the oral epithelium, the main site of involvement in BD, has enriched hBD-2 expression.[23] The BD patients in the present study showed significantly higher *DEFB4* copy numbers than the healthy controls. Such an association between *DEFB4* copy numbers and BD has not been previously reported. However, no significant relationship was observed between genomic copy number variations of *DEFB4* and the various clinical manifestations of BD.

The results obtained from the current study were not consistent with previous research from South Korea, which found no significant correlation between *DEFB4* copy numbers and BD.[24] These contradictory results could be due to several reasons. It is well known that the effect of variation in *β-defensin* genomic copy numbers is regarded as complex, rather than simple, copy number variation; this is difficult to evaluate in small- or moderate-sized case-control studies. As a result, very few good candidate genes have been accurately studied in genomic association trials.[25] The results of Aldhous *et al*. support this observation; in spite of a large sample size, they failed to replicate both the previously reported high and low genomic copy numbers of *DEFB4* among patients with Crohn's disease.[19,26,27] In addition, discrepancies in assay reliability could play a role in explaining differences in the obtained results as well as the difference between the paralog ratio test used in the Korean study and the quantitative PCR assay used in the present study.[24] However, Fernandez-Jimenez *et al*. demonstrated that both techniques can produce comparable *DEFB4* results with the use of optimum DNA normalisation and high-quality genomic DNA.[28] Nevertheless, it is known that *DEFB4* copy numbers vary between ethnicities;[29] this might account for the differences observed between the studied Iraqi and Korean populations.[24] Genetic heterogeneity was observed in the *β-defensin* gene cluster among 67 populations; this may have been due to selection.[30] Such genetic heterogeneity may differ along geographical or racial lines in different populations.

One of the limitations of this study was the small sample size. It is important to note that the current study simply presents the significantly higher counts of mean *DEFB4* genomic copies in BD, drawing attention to a possible pathological association. Further research should focus on the influence of these results on hBD-2 levels both in the peripheral tissues (especially the orogenital mucosa) and serum of BD individuals. Nevertheless, the present study is the first to analyse *DEFB4* in a cohort of healthy Iraqi men and women. The median genomic copy number found among this group was similar to the modal diplotype genomic copy numbers observed in seven different populations.[12] A large-scale multicentre study is recommended to validate the results of the present study and to investigate the functional consequences of these copy number variations in terms of hBD-2 protein expression.

Conclusion

There was an association between BD and *DEFB4* genomic copy numbers among the studied Iraqi subjects, with BD patients having significantly higher *DEFB4* genomic copy numbers than the control group. This suggests that high *DEFB4* genomic copy numbers may be associated with susceptibility to BD. However, no association was found between *DEFB4* genomic copy numbers and the different clinical manifestations of BD. Among the healthy Iraqi control group, the median *DEFB4* genomic copy number was comparable to numbers observed in other populations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

References

1. Sibley C, Yazici Y, Tascilar K, Khan N, Bata Y, Yazici H, et al. Behçet syndrome manifestations and activity in the United States versus Turkey: A cross-sectional cohort comparison. J Rheumatol 2014; 41:1379–84. doi: 10.3899/jrheum.131227.
2. Hatemi G, Seyahi E, Fresko I, Talarico R, Hamuryudan V. Behçet's syndrome: A critical digest of the 2013-2014 literature. Clin Exp Rheumatol 2014; 32:S112–22.
3. Tappuni AR, Tbakhi A, Sharquie KE, Hayani RK, Al-Kaisi A, Lafi A, et al. A comparative study of the genetics of Behcet's disease in Iraq: International collaboration to transfer clinical and laboratory skills to Baghdad medical school and hospitals. Med Confl Surviv 2013; 29:57–68. doi: 10.1080/13623699.2013.765201.
4. Demirseren DD, Ceylan GG, Akoglu G, Emre S, Erten S, Arman A, et al. HLA-B51 subtypes in Turkish patients with Behçet's disease and their correlation with clinical manifestations. Genet Mol Res 2014; 13:4788–96. doi: 10.4238/2014.July.2.8.
5. Gul A, Ohno S. HLA-B*51 and Behçet disease. Ocul Immunol Inflamm 2012; 20:37–43. doi: 10.3109/09273948.2011.634978.
6. Lee YJ, Kang SW, Song JK, Baek HJ, Choi HJ, Bae YD, et al. Associations between interferon regulatory factor-1 polymorphisms and Behcet's disease. Hum Immunol 2007; 8:770–8. doi: 10.1016/j.humimm.2007.06.002.
7. Akman A, Sallakci N, Coskun M, Bacanli A, Yavuzer U, Alpsoy, et al. TNF-alpha gene 1031 T/C polymorphism in Turkish patients with Behcet's disease. Br J Dermatol 2006; 155:350–6. doi: 10.1111/j.1365-2133.2006.07348.x.
8. Groth M, Szafranski K, Taudien S, Huse K, Mueller O, Rosenstiel P, et al. High-resolution mapping of the 8p23.1 beta-defensin cluster reveals strictly concordant copy number variation of all genes. Hum Mutat 2008; 29:1247–54. doi: 10.1002/humu.20751.

9. Hollox EJ, Armour JA, Barber JC. Extensive normal copy number variation of a beta-defensin antimicrobial-gene cluster. Am J Hum Genet 2003; 73:591–600. doi: 10.1086/378157.

10. Schneider JJ, Unholzer A, Schaller M, Schäfer-Korting M, Korting HC. Human defensins. J Mol Med (Berl) 2005; 83:587–95. doi: 10.1007/s00109-005-0657-1.

11. Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: More than just microbicidal. Trends Immunol 2002; 23:291–6. doi: 10.1016/S1471-4906(02)02246-9.

12. Hollox EJ. Copy number variations of beta-defensins and relevance to disease. Cytogenet Genome Res 2008; 123:148–55. doi: 10.1159/000184702.

13. International Study Group for Behçet's Disease. Criteria for diagnosis of Behçet's disease. Lancet 1990; 335:1078–80. doi: 10.1016/0140-6736(90)92643-V.

14. Scherrer MA, de Castro LP, Rocha VB, Pacheco L. [The dermatoscopy in the skin pathergy testing: Case series in patients with suspected Behçet's Disease.] Rev Bras Reumatol 2014; 54:494–8. doi: 10.1016/j.rbr.2014.06.003.

15. National Center for Biotechnology Information. Homo sapiens beta-defensin 2 (DEFB2) gene, complete cds: GenBank AF040153.1. From: www.ncbi.nlm.nih.gov/nuccore/AF040153 Accessed: Jun 2015.

16. Jaradat SW, Hoder-Przyrembel C, Cubillos S, Krieg N, Lehmann K, Piehler S, et al. Beta-defensin-2 genomic copy number variation and chronic periodontitis. J Dent Res 2013; 92:1035–40. doi: 10.1177/0022034513504217.

17. Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, Rodijk-Olthuis D, et al. Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet 2008; 40:23–5. doi: 10.1038/ng.2007.48.

18. Janssens W, Nuytten H, Dupont LJ, Van Eldere J, Vermeire S, Lambrechts D, et al. Genomic copy number determines functional expression of {beta}-defensin 2 in airway epithelial cells and associates with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 182:163–9. doi: 10.1164/rccm.200905-0767OC.

19. Bentley RW, Pearson J, Gearry RB, Barclay ML, Mckinney C, Merriman TR, et al. Association of higher DEFB4 genomic copy number with Crohn's disease. Am J Gastroenterol 2010; 105:354–9. doi: 10.1038/ajg.2009.582.

20. Jansen PA, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jonph GJ, et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS One 2009; 4:e4725. doi: 10.1371/journal.pone.0004725.

21. Zhou XJ, Cheng FJ, Lv JC, Luo H, Yu F, Chen M, et al. Higher DEFB4 genomic copy number in SLE and ANCA-associated small vasculitis. Rheumatology (Oxford) 2012; 51:992–5. doi: 10.1093/rheumatology/ker419.

22. Sayinalp N, Ozcebe OI, Ozdemir O, Haznedaroğlu IC, Dündar S, Kirazli S. Cytokines in Behçet's disease. J Rheumatol 1996; 23:321–2.

23. Joly S, Organ CC, Johnson GK, McCray PB Jr, Guthmiller JM. Correlation between beta-defensin expression and induction profiles in gingival keratinocytes. Mol Immunol 2005; 42:1073–84. doi: 10.1016/j.molimm.2004.11.001.

24. Park JJ, Oh BR, Kim JY, Park JA, Kim C, Lee YJ, et al. Copy number variation of β-defensin genes in Behçet's disease. Clin Exp Rheumatol 2011; 29:S20–3.

25. Olsson LM, Holmdahl R. Copy number variation in autoimmunity: Importance hidden in complexity? Eur J Immunol 2012; 42:1969–76. doi: 10.1002/eji.201242601.

26. Fellermann K, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, et al. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn's disease of the colon. Am J Hum Genet 2006; 79:439–48. doi: 10.1086/505915.

27. Aldhous MC, Abu Bakar S, Prescott NJ, Palla R, Soo K, Mansfield JC, et al. Measurement methods and accuracy in copy number variation: Failure to replicate associations of beta-defensin copy number with Crohn's disease. Hum Mol Genet 2010; 19:4930–8. doi: 10.1093/hmg/ddq411.

28. Fernandez-Jimenez N, Castellanos-Rubio A, Plaza-Izurieta L, Gutierrez G, Irastorza I, Castaño L, et al. Accuracy in copy number calling by qPCR and PRT: A matter of DNA. PLoS One 2011; 6:e28910. doi: 10.1371/journal.pone.0028910.

29. Li J, Yang T, Wang L, Yan H, Zhang Y, Guo Y, et al. Whole genome distribution and ethnic differentiation of copy number variation in Caucasian and Asian populations. PLoS One 2009; 4:e7958. doi: 10.1371/journal.pone.0007958.

30. Hardwick RJ, Machado LR, Zuccherato LW, Antolinos S, Xue Y, Shawa N, et al. A worldwide analysis of beta-defensin copy number variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. Hum Mutat 2011; 32:743–50. doi: 10.1002/humu.21491.